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Biological Control of Soilborne Diseases on Tomato, Potato and Black Pepper by Selected PGPR in the Greenhouse and Field in Vietnam

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Bacterial wilt, Fusarium wilt and Foot rot caused by *Ralstonia solanacearum*, *Fusarium oxysporum*, and *Phytophthora capsici* respectively, continue to be severe problems to tomato, potato and black pepper growers in Vietnam. Three bio-products, *Bacillus vallismortis* EXTN-1 (EXTN-1), *Bacillus* sp. and *Paenibacillus* sp. (ESSC) and *Bacillus substilis* (MFMF) were examined in greenhouse bioassay for the ability to reduce bacterial wilt, fusarium wilt and foot rot disease severity. While these bio-products significantly reduced disease severities, EXTN-1 was the most effective, providing a mean level of disease reduction 80.0 to 90.0% against bacterial wilt, fusarium wilt and foot rot diseases under greenhouse conditions. ESSC and MFMF also significantly reduced fusarium wilt, bacterial wilt and foot rot severity under greenhouse conditions. Bio-product, EXTN-1 with the greatest efficacy under greenhouse condition was tested for the ability to reduce bacterial wilt, fusarium wilt and foot rot under field condition at Song Phuong and Thuong Tin locations in Ha Tay province, Vietnam. Under field condition, EXTN-1 provided a mean level of disease reduction more than 45.0% against all three diseases compared to water treated control. Besides, EXTN-1 treatment increased the yield in tomato fruits 17.3% than water treated control plants.

Keywords : biological control, bacterial wilt, fusarium wilt, EXTN-1, PGPR-mediated ISR and tomato, foot rot

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the economically important diseases and is of world-wide distributed, having been reported in at least 32 countries. It causes 80% loss in severe cases (McGrath et al., 1987; Malhotra and Vashistha, 1993). *Ralstonia solanacearum* is another important soilborne bacterial plant pathogen with a worldwide distribution and a wide host range of more than 200 species in 50 families (Hayward, 1991). It causes a lethal bacterial wilt disease of diverse plants viz., tomato, potato, eggplant, pepper, tobacco, banana, chilli, and peanut (French and Sequeira, 1970). *R. solanacearum* invades the xylem vessels of roots and disseminates into the stem where multiplies and wilts by excessive exopolysaccharide production (Schell, 2000). In Vietnam, tomato, potato and black pepper among economically important crops are high value export crops. However, the production of these crops is remarkably being reduced by economically important soilborne bacterial and fungal pathogens. The weather conditions also influence the pest development in Vietnam (Truong et al., 2008; Doan and Nguyen, 2006). In Vietnam, potato is as a winter crop, the second most important food crop after maize and some 30,000-35,000 hectares of potatoes are cultivated. However, the average yield is only about 12 ton/h. Black pepper is among the top ten export items of Vietnam by value and provides much needed income for subsistence farmers. In 2004, Vietnam had a growing area of black pepper of about 52,500 ha and a productive of nearly 90,000 ton (Ton, 2005). Unfortunately, several diseases hamper black pepper production in Vietnam, particularly by foot rot caused by *Phytophthora capsici* (Nguyen, 2002; Dreath and Sendall, 2004). *P. capsici* Leonian is a soilborne heterothallic oomycete with two mating types, A1 and A2. Both mating types have been reported to coexist in several black pepper areas in Vietnam (Nguyen et al., 2006) and it causes an estimated annual loss of 15-20% (Drenth and Sendall, 2004). Control of these soilborne diseases is mainly through chemical soil fumigation and resistant cultivars. However, the broad-spectrum biocides used to fumigate soil before planting are environmentally damaging. Thus, the management of these diseases with chemical measures has proved impractical mainly because of the appearance of fungicide-resistant strains (Hide et al., 1992).

The use of chemicals was widely resorted to in order to achieve high levels of disease suppression. However, the persistent, injudicious use of chemicals has been discouraged owing to their effects on nontarget organisms and due to the

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undesirable changes they inflict upon the environment. Many of the chemicals are also too expensive for the resource-poor farmers of Asia. Therefore, biological control assumes special significance in being an ecofriendly and cost-effective strategy for disease management. In recent years, biological control has emerged as an important alternative in managing soilborne plant diseases (Whipps, 1997). Plant growth-promoting rhizobacteria (PGPR) are free-living soil-borne bacteria that colonize the rhizosphere and enhance the growth of plants (Kloepper et al., 1980). PGPR that have been successful in promoting the growth of crops such as canola, soybean, lentil, pea, wheat and radish have been isolated (Kloepper et al., 1988; Chanway et al., 1989; Glick et al., 1997; Timmusk et al., 1999). The severity of plant diseases caused by a variety of soilborne pathogens has been reduced by rhizobacterial strains of *Pseudomonas*, *Burkholderia*, and *Bacillus* spp. (Weller, 1988). In this study, three bio-products, EXTN-1 (*Bacillus vallismortis*), ESSC (*Bacillus* sp. and *Paenibacillus* sp.) and MFMF (*Bacillus substilis*) were evaluated against soilborne diseases of bacterial wilt, Fusarium wilt (tomato and potato) and foot rot (black pepper) under greenhouse and field conditions at Song Phung, Hoai and Thanh Tin, Ha Tay province of Vietnam.

Materials and Methods

Microorganisms and Experimental site. Greenhouse and field experiments were conducted at Hoai Duc and Thanh Oai of Ha Tay province (located at 21°09' N Lat105° 25' E Long 16.4 m) in Vietnam. Three bio-products, EXTN-1 (Park et al., 2001), ESSC (*Bacillus* sp. and *Paenibacillus* sp. at concentration of 1×10^7 cfu/g) and MFMF (*Bacillus substilis* at concentration of 1×10^7 cfu/ml) were used in greenhouse and field experiments. MFMF bio-product was kindly provided from Institute of soil science and fertilizer, Hanoi. ESSC product was obtained from Daekyung vina JVC, Fertilizer Company in Vietnam. Bacterial and fungal pathogens used for the challenge inoculation were *Ralstonia solanacearum*, *Fusarium oxysporum* and *Phytophthora capsici* causal agents of bacterial wilt, fusarium wilt and foot rot diseases, respectively. The bacterial and fungal pathogens were maintained on sucrose peptone agar (SPA) plates at 28°C and potato dextrose agar (PDA) plates at 24°C, respectively. In this study, disease susceptible tomato (Poland and PT18 varieties), potato (Ackersegen and Diamand varieties) and black pepper (Tieu Se variety) obtained from Plant Protection Research Institute of Vietnam Academy of Agricultural Science were used.

Preparation of pathogen inoculum. Bacterial pathogen, *R. solanacearum* was grown in sucrose peptone broth (SPB) at 30°C for 24 h at 150 rpm. Bacterial suspension of *R. solanacearum* was adjusted to 2×10^7 cfu/ml by counting with hemacytometer. Plant pathogens, *P. capsici* and *F. oxysporum* were grown on V8 juice agar and potato dextrose agar (PDA) medium for two weeks, respectively. Zoosporangia of *P. capsici* and conidial spores *F. oxysporum* were scrapped from medium by adding sterile water and they were adjusted to 10^4 sporangia/ml and 10^6 conidia/ml, respectively, by counting with hemacytometer.

Greenhouse experiment. Tomato seeds were surface-sterilized with 2% sodium hypochlorite for 2 min, washed thoroughly with sterilized water, and planted into pots of sterilized soil. After 4 weeks, before transplanting, seedling roots were dipped into diluted bacterial suspensions (10^6 cfu/ml concentration) of EXTN-1, ESSC, MFMF and water for 1 h. Then, seedlings were transplanted into pots containing experimental seedbed soil and grown in the greenhouse at 25-35°C. After 2 days of transplanting, each 30 ml of bacterial suspension of *R. solanacearum* (2×10^7 cfu/ml) and conidial suspension of *F. oxysporum* (10^5 spores/ml) were soil drenched separately. In the experiments on potato, seed tubers of Ackersegen and Diamand varieties were given treatment as described above to tomato seedlings. In the black pepper experiment, disease-free single node (approximately 8 cm length) cuttings were surface-sterilized with 2% sodium hypochlorite for 2 min, washed thoroughly with sterilized water. Before planting, black pepper cuttings were dipped into bacterial suspension of EXTN-1, ESSC, MFMF and water for 1h. Then, they were planted into pots containing experimental seedbed soil and grown in the greenhouse at 25-35°C. After 2 days, a 30 ml of zoosporangia suspension of *P. capsici* (10^4 sporangia/ml) was soil drenched around the stem. Subsequent applications of bacterial suspension of EXTN-1, ESSC, MFMF and water were given as foliar spray at 7, 15 and 30 days after planting. For greenhouse experiment, the soil was obtained from healthy tomato fields in Hanoi, near Plant Protection Research Institute campus. The soil was a clay loam, pH 6.8, and was autoclaved before use. All greenhouse experiments were conducted for three times. There were four control treatments in each experiment: seedlings that were treated with bacterial suspension of EXTN-1 (Control 1); seedlings that were watered with bacterial suspension of ESSC (Control 2); seedlings that were applied with bacterial suspension of MFMF (Control 3); and seedlings that received only H_2O (Control 4). Each treatment contained 20 plants. Disease incidence was rated at 60 days after treatment (<20%-resistant (R); 21-40%-moderately resistant (MR); 41-60%-moderately susceptible (MS); 61-85%->86%-highly susceptible (HS).

Field experiment. Field experiment of tomato treatment was conducted at Song Phuong, Hoai Duc during summer season in 2007. In 2006, field experiment of potato treatment was conducted at Thuong Tin, Ha Tay province during winter season. All field experiments on tomato, potato and black pepper were not included artificial challenge inoculation. For tomato and potato experiments, each plot was 5 m in length and 2 m in width in one hectare and a number of sixty seedlings were planted in each plot. The bacterial suspension of EXTN-1 was diluted (10^6 cfu/ml) with water, mixed evenly into the organic fertilizer, and applied to the soil. Tomato and potato transplantings were conducted 7 days later. Except for the application of pesticides, standard agronomic practices were conducted to raise the crop. For black pepper, modelling black pepper field with 1, 3 and 4-years-old plants was used. Subsequent applications of foliar spray with bacterial suspension of EXTN-1 were given at 7, 15 and 30 days after transplantation. Field trial included the two treatments : Suspension of EXTN-1 and a water control. The experimental design was a complete randomized block. The number of wilted plants was recorded at 60 and 120 days for tomato and 120 days for potato. The disease incidence on black pepper was recorded at 6 and 14 months after treatment. Disease incidence was calculated with the following formula : 100x(number of wilted plants per plot/total number of plants per plot). Plant growth promotion of tomato was determined in terms of yield. Tomato fruits were harvested three times in each plot and the cumulative yields were calculated. All fruits of tomato were harvested randomly from ten plants of each plot of each treatment and the total yield per plant was obtained. The yield of each plot was calculated as the percentage of yield per plant. Likewise, total yield was calculated for one hectare.

Statistical analysis. In this study, all data were analyzed with JMP (a PC-version of SAS) (SAS Institute. 1995) software. Significant differences in treatments were determined using Student's T test as LSD at P=0.05.

Results

Root drench and foliar application of bio-products significantly reduced severity of bacterial wilt on tomato and potato, fusarium wilt on tomato and foot rot on black pepper under greenhouse conditions (Table 1-3). There were upto 91% and 85.5% reduction in severity of bacterial wilt was observed in PT 18 and Poland varieties, respectively treated with EXTN-1, ESSC and MFMF. More than 80.0% reduction in severity of fusarium wilt was observed in both variety of tomato (Table 1). In potato, bacterial wilt disease was significantly reduced on potato varieties Ackersegen (81.9 to 87.3%) and Diamand (87.0 to 92.8%) treated with three bio-products compared to control treatment under greenhouse condition (Table 2). Application of three bio-products on black pepper reduced the disease incidence of foot rot by 26.6 to 40% under greenhouse condition (Table 3). In greenhouse study, reduction in intensity of disease provided by three bio-products varied with pathogen tested. In field, EXTN-1 treatment significantly

Table 1. Tomato plant protection against *Ralstonia solanacearum* and *Fusarium oxysporum* by selected PGPR products under greenhouse condition

Treatments	Diseased plants (%)			
	R. solanacearum		*F. oxysporum*	
	PT 18	Poland	PT 18	Poland
Control	89.5	98.3	92.0	98.2
EXTN-1	8.0*	14.2*	7.5*	13.4*
ESSC	10.2*	17.0*	8.5*s	18.2*
MFMF	11.3*	20.2*	10.0*	20.0*
LSD(P=0.05)	4.6	3.6	3.5	3.7

Mean of disease incidence of three replications in percentage; Each replication consisted twenty plants.
*=significantly different (P=0.05) according to Student's Least Significant Difference (LSD) test.

Table 2. Potato plant protection against Bacterial wilt by selected PGPR products under greenhouse condition

Treatments	Diseased plant (%)	
	Ackersegen	Diamand
Control	96.3	84.5
EXTN-1	12.2*	6.1*
ESSC	15.2*	9.1*
MFMF	17.4*	11.0*
LSD (p=0.05)	4.0	3.8

Mean of disease incidence of three replications in percentage; Each replication consisted twenty plants.
*=significantly different (P=0.05) according to Student's Least Significant Difference (LSD) test.

Table 3. Black pepper protection against *Phytophthora capsici* by selected PGPR products under greenhouse condition

Treatments	Diseased plant (%)
Control	60.0
EXTN-1	36.0*
ESSC	40.0*
MFMF	44.0*
LSD (p=0.05)	5.6

Mean of disease incidence of three replications in percentage; Each replication consisted twenty plants.
*=significantly different (P=0.05) according to Student's Least Significant Difference (LSD) test.

Table 4. Tomato yield increase and production against *R. solanacearum* and *F. oxysporum* by treatment of *Bacillus vallismortis* EXTN-1 at Song Phuong, Hoai Duc, Ha Tay province, Vietnam

Treatment	Diseased plant (%)				Yield (Ton/ha)
	R. solanacearum		*F. oxysporum*		
	60 days	120 days	60 days	120 days	
Control	11.3	15.3	6.7	10.7	41.890
EXTN-1	5.3*	8.0*	3.3*	6.0*	50.645
LSD(*P*=0.05)	2.6	2.6	2.0	2.6	-

Mean of disease incidence of three replications in percentage; Each replication consisted sixty plants.
*=significantly different (*P*=0.05) according to Student's Least Significant Difference (LSD) test.

reduced disease incidence of bacterial wilt, fusarium wilt and foot rot as well as it enhanced the yield in tomato plants. The field experiments were conducted during winter potato and summer tomato seasons at Thuong Tin and Hoic Duc of Hay Tay province, Vietnam. In variety PT 18, disease incidence of Bacterial wilt was reduced 53.0% and 47.7% at 60 and 120 DAT(day after treatment) respectively, compared to control (Table 4). Disease protection on tomato against fusarium wilt was observed 49.3% and 43.9% at 60 and 120 DAT, respectively. Furthermore, EXTN-1 treatment increased yield (17.3%) in tomato compared to control (Table 4). By EXTN-1treatment, disease reduction of bacterial wilt and fusarium wilt on potato variety, Diamand was observed to 48.7 and 53.2%, respectively (Table 5). On black pepper at 6 month after treatment with EXTN-1, disease incidence of foot rot was recorded 54.0, 45.6 and 44.7% on 1, 3 and 4 years old plants, respectively. And also, Foot rot disease was controlled to 37.5, 37.8 and 34.8% at 14 month after treatment on 1, 3 and 4-years-old plants, respectively (Fig. 1).

Table 5. Potato plant protection against *R. solanacearum* and *F. oxysporum* by selected PGPR product under field condition during winter season in Vietnam

Treatment	Diseased plant (%)	
	R. solanacearum	*F. oxysporum*
Control	15.6	21.8
EXTN-1	8.0*	10.2*
LSD (*P*=0.05)	1.3	1.4

Mean of disease incidence of three replications in percentage; Each replication consisted sixty plants.
*=significantly different (*P*=0.05) according to Student's Least Significant Difference (LSD) test.

Discussion

The results indicated that bio-products of PGPR provided significant disease protection against soilborne bacterial and fungal pathogens under greenhouse and field conditions. While bio-products, ESSC and MFMF afforded significant protection against *R. solanacearum, F. oxysporum* and *P. capsici*, EXTN-1 was the best at controlling diseases. Bio-product, EXTN-1stood out in terms of performance since it reduced the intensity of three diseases tested and thus it was chosen for field study. In previous studies, EXTN-1 has been reported to be involved in induction of systemic resistance on tomato, potato and rice against bacterial, fungal and viral pathogens (Park et al., 2006a, b; Park et al., 2007). Therefore, the present study suggests that indirect mechanism (ISR) of EXTN-1 might be accounted for significant role in disease control against tested soilborne

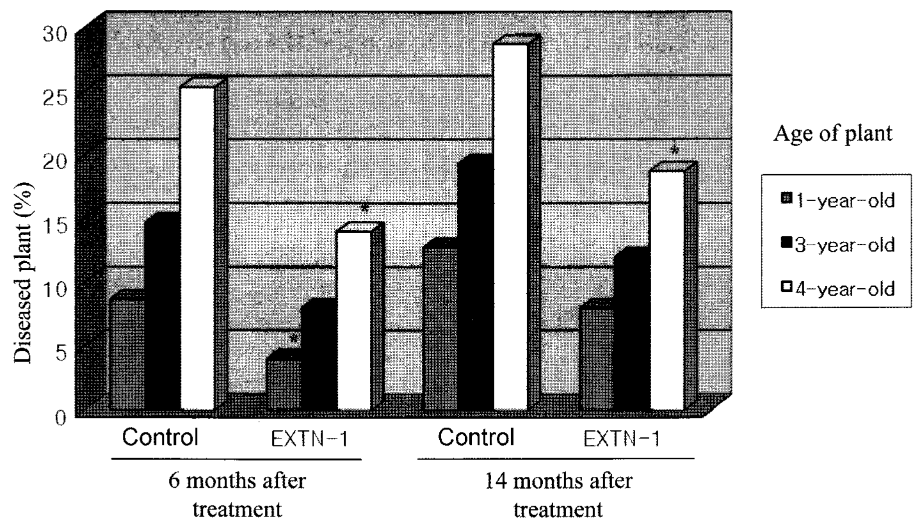

Fig. 1. Black pepper production against foot rot caused *P. capsici* by treatment of *Bacillus vallimortis* EXTN-1 under field condition.
*= significantly different (*P*=0.05) according to student's Least Significant Difference (LSD) test.

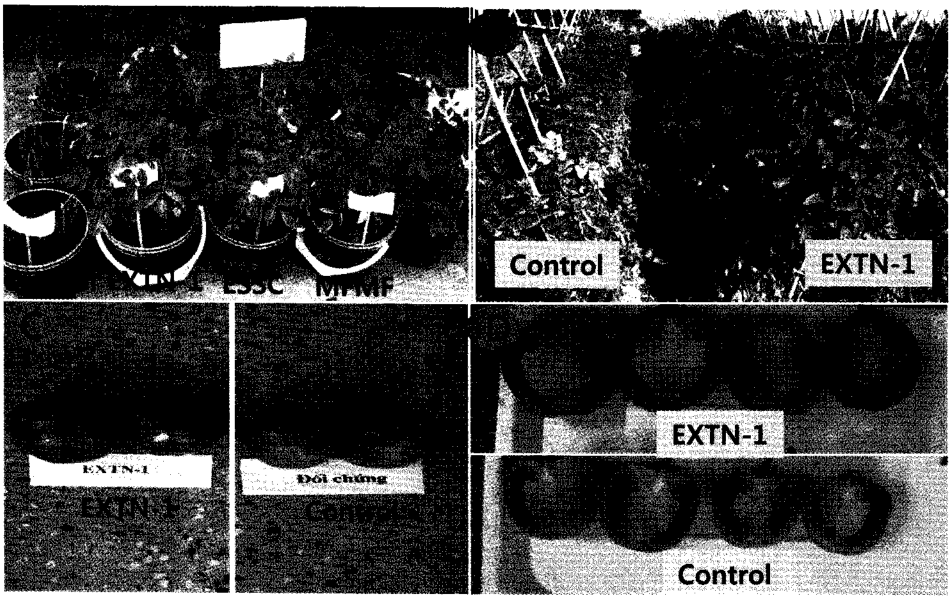

Fig. 2. Plant growth promotion and control of disease incidence on tomato by PGPR strains under greenhouse and field conditions (A: control of disease incidence of bacterial wilt caused by *R. solanacearum* by treatment with PGPR strains compared to water treated control plants; B: enhancement of green pigmentation in leaves; C: enlargement of furit size; D: increment of fleshy part by EXTN-1 treatment compared to water treated control.

pathogens. EXTN-1 (*B. vallismortis*) tested in this study in controlling disease incidence caused by fungal and bacterial pathogens on different plants is highly desirable from the point of view of using formulation of antagonist mixtures (Domenech et al., 2006; de Boer et al., 2003; Raupach and Kloepper, 1998). In field condition, tested EXTN-1bio-product on different plants against different pathogens at different sites resulted in 34 to 54% protection on tomato, potato and black pepper against disease incidence of bacterial wilt, fusarium wilt and foot rot during summer and winter seasons. In this case, EXTN-1 (*B. vallismortis*) treatment differs from the concept of mixture of antagonists controlling different pathogens requiring different conditions of temperature, humidity and pH for plant root system colonization (Janisiewicz, 1996). Moreover, significant disease control on tomato varieties, potato varieties and black pepper by EXTN-1 treatment indicates that the effects of genotype of different varieties did not influence on EXTN-1.

Advancing from greenhouse trials to field trials is an important step toward the goal of practical applications of ISR elicited by PGPR (Knudsen et al., 1997; Kloepper et al., 2004). There are many examples over the control of soilborne disease by 56% using Bacillus strains in greenhouse (Lemessa and Zeller, 2007) or 30 to 65% reduction at field level with inconsistence performance (Larkin and Fravel, 1998). In a study, Jubina and Girija (1998) reported the highest diseases suppression of foot rot of black pepper in the *in vivo* biological control assay using *Bacillus* sp. in nursery plants. However, there are only few examples of efficient biocontrol in the field conditions in Chile, Bangladesh and China against *R. solanacearum* on tomato and potato (Ciampi-Panno et al., 1989; Guo et al., 2004; Jinnah et al., 2002). Very recently, effective control of *P. capsici* in field application has been conducted with bio-product containing chitinolytic bacteria (Kim et al., 2008).

In this study, we estimated plant growth promotion on tomato along with bio control activity by treatment with EXTN-1 under field condition. Impact of root inoculation and foliar spray with EXTN-1 explored on some quality parameters in field experiments on tomato viz., increased fruit size, fleshy part of tomato, green pigmentation in leaf and age of the plant (Fig. 2) along with yield. The yield increase was recorded 17.3% compared to water treated control. Similarly, Guo et al. (2004) has reported the increased yield in tomato with formulated PGPR products in field trials. In PGPR formulations, the shelf life of a biocontrol product is mainly depended on the characteristics of the biocontrol agent itself. In this case, bioproduct, EXTN-1 has assumed to have long shelf life due to accounted for protection of crops upto harvesting stage. And also, one of the most important problems in biocontrol using microbial products is the storage time of living microbes. In a previous study, Vidhyasekaran et al. (1997) has reported the problems in storing unformulated bacterial suspensions. By EXTN-1 treatment, tomato plant survival time was recorded to be increased (data not shown) and this feature might account for increased yield in tomato. Violante and Portugal (2007) reported that Bacillus strains have positive effects on tomato fruit quality attributes, particularly on size and texture. And, in field experiments, plants were supplied with the proper amounts of all nutrients since facilitating plant nutrition could be the mechanism by which PGPR enhance crop yield and fruit size (Bar-Ness et al., 1992; Richardson, 2001).

In previous study, EXTN-1 (*B. vallismortis*) had been observed to induce systemic resistance against various pathogens (Park et al., 2001). Ahn et al. (2002) has reported that the major mechanisms by which EXTN-1 bring about disease suppression in crops is by the induction of systemic resistance to the host plant. And, these resistance mechanisms of EXTN-1 have been proven to be effective against bacterial, fungal and viral pathogens of different crops (Ahn et al., 2002; Park et al., 2006a; 2006b, 2007).

In this study, EXTN-1 treatment has played significant role on disease control of soilborne bacterial and fungal pathogens as well as plant growth promotion under both greenhouse and field conditions in Vietnam. The results from our studies suggests that application of *B. vallimortis* EXTN-1 can provide non-specific disease resistance as well as plant growth promotion on different host plants.

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